Pharmacology Biochemistry & Behavior, Vol. 25, pp. 29–33, 1986. © Ankho International Inc. Printed in the U.S.A. 0091-3057/86 $3.00 + .00

Alterations of Amphetamine Elicited Perseveration and Locomotor Excitation Following Acute and Repeated Stressor Application[1]

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Received 27 December 1985

HAHN, B., R. M. ZACHARKO AND H. ANISMAN. *Alterations of amphetamine elicited perseveration and locomotor excitation following acute and repeated stressor application.* PHARMACOL BIOCHEM BEHAV **25**(1) 29–33, 1986.—The effects of acute and repeated stressor application on amphetamine-induced Y-maze perseveration and locomotor activity were assessed. When the stimulus context associated with an acute stressor (restraint or restraint plus shock) was distinctively different from that in which an amphetamine test was conducted 72 hr afterward, neither perseveration nor locomotor excitation were augmented. However, following three restraint sessions the amphetamine elicited perseveration was enhanced. With a more protracted regimen applied over 15 days the augmented perseveration was absent, whereas the amphetamine-provoked motor excitation was increased. While stimulus factors have been shown to be fundamental, it is provisionally suggested that the stressor induced enhancement of amphetamine-elicited perseveration is influenced by sensitization processes. However, the sensitization is apparent only under some stress regimens, and the behavioral expression of the sensitization may be obfuscated if the stressor is too severe. Furthermore, it appears that the mechanisms operative in enhancing the stressor provoked amphetamine motor excitation are independent of those which subserve the augmented perseveration.

d-Amphetamine Stress Perseveration Locomotor activity

ALTHOUGH stressor-provoked neurochemical changes are relatively transient, re-exposure to limited aversive stimulation or to cues previously associated with a stressor engender marked variations of norepinephrine (NE) and dopamine activity [1, 6, 7]. Likewise, the behavioral effects (e.g., stereotypy, locomotor activity and circling) of stimulant drugs may be enhanced in previously stressed animals [4, 5, 7, 11, 12]. Since stressors and amphetamine have several common effects on NE and DA activity [2,4], coupled with the findings that stressors augment the neurochemical consequences of the drug [16], it was suggested that stressors induce sensitization of the mechanisms associated with neurochemical lability, thereby enhancing the behavioral response to subsequent drug treatment [3].

Like the motor excitation and stereotypy, stimulus perseveration (i.e., the tendency to enter successively two arms of a Y-maze in a free choice exploration task) elicited by amphetamine was enhanced by prior exposure to inescapable shock [3]. Contrary to the stereotypy, however, several days after stressor application the enhanced perseveration was apparent only if testing was conducted in a stimulus context similar to that in which shock had initially been delivered. Thus, it was suggested that conditioning processes may be fundamental to the elicitation of perseveration which is influenced by NE neuronal activity [10], whereas the proactive effects of stressors on DA mediated behaviors such as stereotypy and motor excitation, reflects the sensitization of neurochemical processes [3].

An alternative accounting for the differential effects of stressors may be related to the fact that perseveration was assessed after a single stress session [3], whereas stereotypy and locomotor activity were determined after repeated stressor application [4, 5, 8, 11]. Indeed, it was recently reported [8] that amphetamine-elicited motor excitation was enhanced to a greater degree after 10 shock sessions than after a single session. Furthermore, it was demonstrated that following chronic stressor application a pronounced increase of NE synthesis is provoked, well beyond that induced by an acute stressor [15], thereby preventing the NE depletion ordinarily associated with acute stress [9,13]. Moreover, upon re-

[1]Supported by Grants A9845 and A1087 from the Natural Sciences and Engineering Research Council of Canada.

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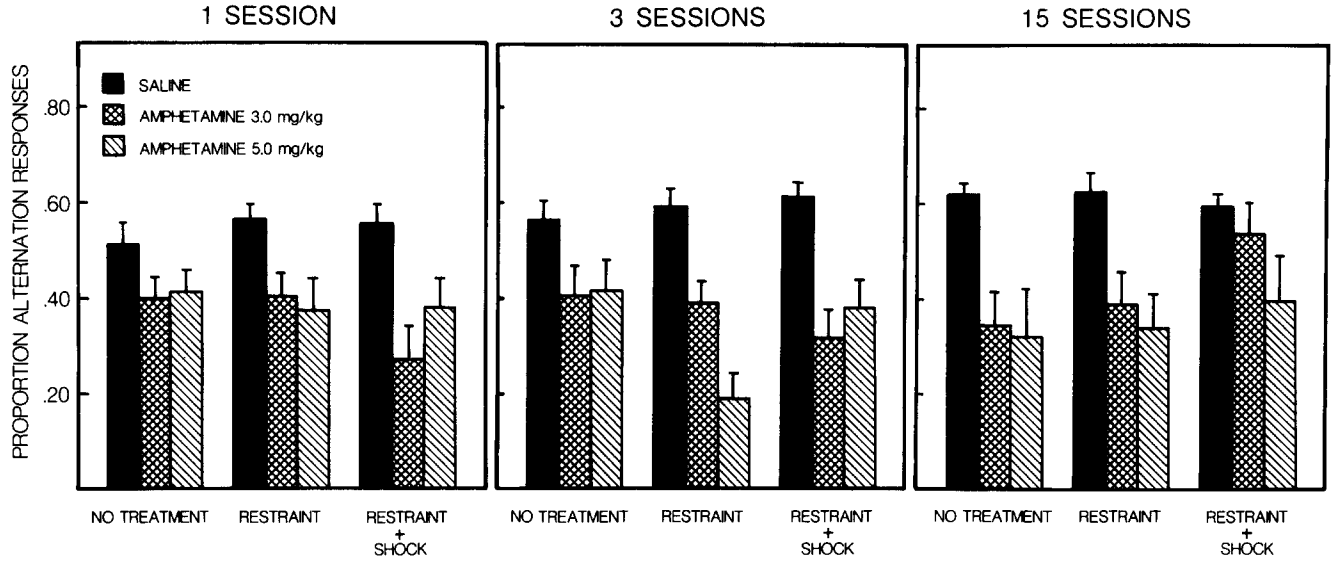

FIG. 1. Mean (±S.E.M.) proportion of alternation responses in mice as a function of the stressor (restraint, restraint plus shock or no treatment), the number of such sessions to which mice were exposed (1, 3, or 15), and the drug treatment administered 72 hr afterward (amphetamine 3.0 or 5.0 mg/kg or saline).

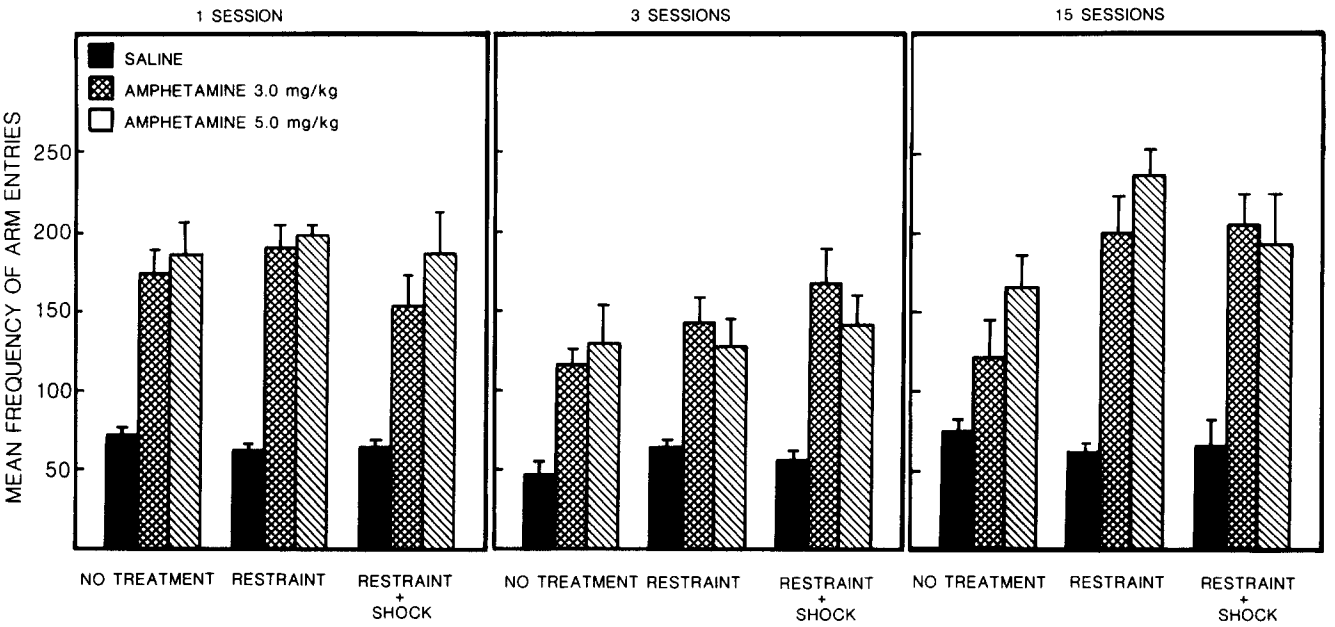

FIG. 2. Mean (±S.E.M.) frequency of arm entries emitted by animals that received no treatment, restraint or restraint plus shock on either 1, 3 or 15 successive days. Mice were tested after treatment of amphetamine (3.0 or 5.0 mg/kg) or saline.

exposure to the stressor or to cues associated with the chronic stressor, NE activity and levels were increased [9]. Thus, it is conceivable that NE variations associated with a chronic stressor may augment amphetamine elicited perseveration to a greater extent than would acute shock. The present investigation assessed the effects of acute and repeated stressor application on amphetamine elicited perseveration in a Y-maze alternation task.

METHOD

A total of 129, 134 and 87 naive, male CD-1 mice (Charles River, Canada) were represented in the three experiments. Mice were housed in groups of 5 in white polypropylene cages and were permitted a 14 day period of acclimitization to the laboratory before serving as experimental subjects.

The apparatus was the same as that previously described

[3]. Shock was delivered in semicircular, clear, Plexiglas restraining tubes which measured 4.9 cm in length. The mouse's tail, which protruded from the restraining tube, was fastened to a Plexiglas plate. Two aluminum foil electrodes, 0.5 cm in width, wrapped about the base and mid section of the tail, were connected to a shock generator which could deliver current of 150 μA, AC.

Alternation/perseveration was assessed in symmetrical black Plexiglas Y-mazes, with arms 22.0×9.0×15.0 cm. Infrared photodetector cells located within each of the arms monitored the location of the animal. The floor of each maze was comprised of 0.32 cm stainless steel rods spaced 1.0 cm apart. Red Plexiglas roofs reduced illumination in the mazes. Each maze was housed in sound attenuating chambers. Thus, the characteristics of the mazes, as well as the surrounding in which they were kept, were distinctive from that in which shock was delivered.

Three experiments assessed the effects of 1, 3 or 15 stress sessions on amphetamine-induced perseveration. In each experiment mice of one group were placed in the restraining apparatus for 1.1 hr, during which they received 60 tail-shocks (2 sec, 150 μA) at 60 sec intervals. The second group was also restrained for a 1.1 hr, but shock was not delivered. The third group of mice were handled, but neither restrained nor shocked. In the first experiment (n=13–15/group) the treatments were applied on only a single occasion, while in the second (n=13–15/group) and third experiment (n=8–10/group) the treatments were administered on each of 3 and 15 consecutive days, respectively. Following the last treatment session mice were returned to their home cages and left undisturbed for 72 hr. On the test day mice were individually placed in the Y-maze for a 6 min adaptation period. Immediately thereafter mice received intraperitoneal injection of either d-amphetamine sulfate (Smith Kline & French) (3.0 or 5.0 mg/kg in a volume of 10 ml/kg) or saline, and placed in the choice area of the Y-maze 15 min later. The sequence and number of arm entries was recorded over a 15 min period.

RESULTS

Spontaneous alternation (the tendency to enter the least recently visited arm) was determined as a proportion of alternation responses divided by alternation plus nonalternation responses (see [3]). Thus, a sequence of arm entries consisting of 1, 2, *3, 1,* 3, 1, *2, 3* received a score of 4 alternation responses and 2 nonalternation responses (spontaneous alternation score=0.66). In a Y-maze paradigm perseveration, or the tendency to enter the most recently visited arm, is the converse of spontaneous alternation. Accordingly, in the preceding example, a nonalternation is actually a perseverative response. Only those animals that displayed 7 or more arm entries were included in the analysis of alternation and perseveration. Thus, scores contributed by a small number of inactive animals did not influence the results. However, all animals were included in the analysis of the frequency of arm entries.

Alternation/Perseveration

The proportion of alternation responses for each group is shown in Fig. 1. Alternation performance was unaffected when testing was conducted in a context different from that in which the stressor had been applied. Analysis of variance of alternation scores in the initial study indicated that only the Drug treatment influenced performance, $F(2,118)=12.88$, $p<0.01$. As seen in Fig. 1, and confirmed by Newman-Keuls multiple comparisons ($\alpha=0.05$), the alternation tendency was reduced following both the 3.0 and 5.0 mg/kg doses of amphetamine.

The response profile seen following 3 stress sessions was markedly different from that seen after a single session. Analysis of variance of the alternation scores revealed a significant Shock treatment × Drug interaction, $F(4,122)=2.45$, $p<0.05$. Newman-Keuls multiple comparisons ($\alpha=0.05$) of the simple main effects indicated that in nonstressed animals both doses of amphetamine reduced the alternation tendency. In mice that received 3 days of restraint the effect of the low dose of amphetamine (3.0 mg/kg) was not enhanced further; however, the reduction of alternation induced by the 5.0 mg/kg dose was significantly more pronounced. Contrary to the effects of the restraint treatment, exposure to restraint plus shock did not affect amphetamine-elicited perseveration.

Exposure to a stressor on 15 successive days was without effect on amphetamine elicited perseveration (see Fig. 1). Analysis of variance of the alternation scores confirmed that amphetamine reduced the alternation tendency, $F(2,78)=11.00$, $p<0.01$, but neither the Shock main effect nor the Shock × Drug treatment interaction approached statistical significance (F's<1). The lack of the effect of 15 shock sessions on amphetamine elicited perseveration was clearly unexpected. Nevertheless, the differential effects of 3 and 15 stress sessions was repeated on three separate occassions attesting to the fact that the observed Drug × Restraint treatment interaction was not a spurious one.

Arm Entries

The frequency of arm entries varied as a function of the drug treatment and the number of stress sessions. Following 1 or 3 sessions of restraint/shock the frequency of arm entries was increased by both doses of amphetamine, $F(2,118$ and $2,125)=35.91$ and 27.86, p's<0.01. However, neither restraint nor restraint plus shock influenced the effect of amphetamine. In contrast, following 15 stressor sessions the frequency of arm entries was increased by the Amphetamine treatment, $F(2,78)=37.33$, $p<0.01$, as well as by the Shock treatment, $F(2,78)=4.29$, $p<0.05$. Moreover, the Shock treatment × Amphetamine interaction approached statistical significance, $F(4,78)=2.39$, $p=0.058$. Newman-Keuls multiple comparisons ($\alpha=0.05$) indicated that in nonstressed animals amphetamine treatment significantly increased the frequency of arm entries. In mice that had either been restrained or restrained and shocked over 15 days the locomotor excitation produced by 3.0 mg/kg of amphetamine was enhanced relative to nonstressed animals that received the drug. Likewise, the effects of the 5.0 mg/kg dosage on arm entries was increased in mice that received the repeated restraint treatment.

DISCUSSION

As previously observed [8], acute stressor application did not enhance amphetamine-elicited motor activity, whereas a marked enhancement of amphetamine-provoked motor excitation was evident following chronic exposure to a stressor. In contrast, stimulus perseveration engendered by amphetamine varied as a U-shaped function of the number of stress sessions mice received as well as the type of stressor applied. Whereas restraint plus tail-shock did not affect

amphetamine-elicited perseveration, irrespective of the number of sessions administered, three sessions of restraint enhanced the perseveration tendency. However, neither 1 nor 15 sessions of restraint enhanced perseveration provoked by amphetamine. Since the effects of the stressors on amphetamine-elicited perseveration did not parallel the variations of motor activity it appears that these behaviors are independent of one another and are mediated by different mechanisms [10].

The enhanced amphetamine-elicited perseveration provoked by stressors appears to be dependent on conditioning factors, since the effect was only observed when the context in which the drug test was conducted was similar to that in which animals had previously been shocked [3]. Since three sessions of restraint increased amphetamine-elicited perseveration even when the stress and test environments were different, it is likely that some other process, possibly the sensitization of the neurochemical substrate for perseveration, also contributes to the enhancement of this response style.

The source for the stressor-specific enhancement of amphetamine elicited perseveration is not immediately evident, although several provisional hypotheses can be offered. For instance, the stressor-provoked amine or receptor variations subserving the perseveration are nonmonotonic, such that optimal amine variations occur with a moderate amount of stressor application, and then decline (e.g., development of β-NE receptor subsensitivity with chronic stress; [14]). Alternatively, it could be argued that the amine or receptor variations become progressively more pronounced with repeated stressor application, and are reinduced upon subsequent re-exposure to a stressor or cues associated with that stressor [9]. Hence repeated application of a severe stressor would result in a shift of the amphetamine dose response curve so that the optimal dosage of the drug would have been exceeded. This possibility, however, did not appear to be a tenable one since a subsequent study revealed that 1.0 mg/kg of amphetamine, which is not ordinarily sufficient to induce stimulus perseveration was also ineffective in enhancing perseveration in mice that received the stress treatments.

A third accounting for the U-shaped function is that the conditioning or sensitization of the neurochemical processes subserving the perseveration becomes more pronounced with repeated stressor application, but expression of this response style may be obfuscated owing to other behavioral effects of a relatively protracted stress regimen. In fact, we previously reported that following a single session of a relatively severe stressor (e.g., 360 tailshocks) amphetamine-elicited perseveration was less pronounced than after 60 shocks [1]. It has been suggested that perseveration induced by amphetamine is influenced by the organism's ability to attend to specific environmental cues [10]. Thus, a stressor of moderate severity might result in a sensitization effect and hence increase amphetamine-induced perseveration, whereas the heightened arousal associated with a protracted stressor might disrupt attention and hence minimize perseveration.

It appears likely that several different mechanisms may be fundamental in eliciting the proactive effects of aversive events on stimulant induced behaviors. As indicated earlier, conditioning factors may be important in the enhancement of amphetamine-elicited perseveration [1]. In contrast, amphetamine-induced circling following unilateral 6-OHDA lesions is minimized in animals tested in context similar to that in which they had previously been exposed to a stressor [14]. Thus, while conditioning factors may enhance some amphetamine-elicited behaviors, other behaviors provoked by the drug may be suppressed. It also appears that the perseveration induced by amphetamine in stressed animals may be evident when the stress and test environments are distinctively different from one another. Thus, sensitization processes may contribute to this behavioral style; however, a sensitization-like effect occurs only after a moderate amount of aversive stimulation, and with more protracted or severe stressor application the enhancement of the amphetamine-elicited perseveration is absent. In contrast, the motor excitation associated with amphetamine becomes progressively greater with repeated exposure to a stressor. Taken together, these findings suggest that both conditioning and sensitization processes may contribute to the stress $\times$ amphetamine interaction, and their relative contributions vary with the behavior under examination.

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